Spin-spin coupling constants $^{13}C—^{15}N$ and $^{1}H—^{15}N$ in the investigation of azido-tetrazole tautomerism in a series of 2-azidopyrimidines*

I. A. Khalymbadzha,[a] T. S. Shestakova,[a] S. L. Deev,[a] V. L. Rusinov,[a] O. N. Chupakhin,[a,b] Z. O. Shenkarev,[c] and A. S. Arseniev[c]*

[a] *Ural Federal University, 19 ul. Mira, 620002 Yekaterinburg, Russian Federation. Fax: +7 (343) 374 0458. E-mail: deevsl@yandex.ru*
[b] *I. Ya. Postovsky Institute of Organic Synthesis, Russian Academy of Sciences, 22 ul. S. Kovalevskoi, 620041 Yekaterinburg, Russian Federation. Fax: +7 (343) 374 1189. E-mail: chupakhin@ios.uran.ru*
[c] *M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 16/10 ul. Miklukho-Maklaya, 117997 Moscow, Russian Federation*

A new method was developed for the investigation of an azido-tetrazole equilibrium based on using a complex analysis of $^{13}C—^{15}N$ and $^{1}H—^{15}N$ spin-spin coupling constants. The use of this approach became possible due to the selective inclusion of ^{15}N isotopes into the structures of 2-azidopyrimidines and their cyclic analogs tetrazolo[1,5-*a*]pyrimidines.

Key words: azido-tetrazole tautomerism, tetrazolo[1,5-*a*]pyrimidine, spin-spin coupling constants, NMR spectroscopy, ^{15}N isotope.

Practical interest in synthesis of hetaryl azides is due to their ability to participate in photochemical transformations, which allows one to use their derivatives in biological assays. For example, the 2-azidopurine derivatives are applied as photoaffine labels[1,2] for the determination of binding sites of nucleotides with proteins and establishment of the role of these interactions.

The modification of nucleosides due to the insertion of the azido group into heterocyclic bases is known.[3—5] This approach is promising, because the azido group is capable of transforming into the amino group, and is used in synthetic organic chemistry as well. For example, the synthesis of the hetarylamino derivatives of azines from the corresponding azidoazines was described.[6,7]

Azaaromatic compounds with the azido group in the α-position to the nitrogen atom often demonstrate azido-tetrazole tautomerism, which is widely described in the literature.[8—12] This isomerism is characterized by the ability of the azido group in the heterocyclic derivatives to transform under the action of external factors or spontaneously and reversibly into the corresponding tetrazoles. Azido-tetrazole tautomerism makes it possible to consider tetrazoloazines as cyclic analogs of hetaryl azides.[13,14] The studies in the area of azido-tetrazole equilibrium are necessary for controlling the reactivity of these heterocyclic systems classified as ambidentate polyfunctional compounds. So, depending on the conditions, either the tetrazole cycle, or isomeric azido group, or the azine fragment can undergo chemical transformation. It is important that the direction of the reaction often depends on the form in which the compound reacts. One of the examples of the practical successful use of the azido-tetrazole equilibrium is the hydrogenation of tetrazolo[1,5-*a*]pyridine.[15] Under acidic conditions where the tetrazole form predominates, only the pyridine ring is hydrogenated, whereas the hydrogenation under basic conditions stabilizing azide affords 2-aminopyridine. One of the successful examples for the directed reduction of the tetrazole cycle is the synthesis of the modern antiepileptic drug lamotrigine and its analogs.[16]

It is also important to know parameters of the azido-tetrazole equilibrium for the use of photoaffine labels in the field of molecular biology. This is related to the fact that the azide form is photochemically more active than the tetrazole form.[17] Therefore, the maximum concentration of the azide form is necessary in experiments for the efficient application of the photoaffine label.

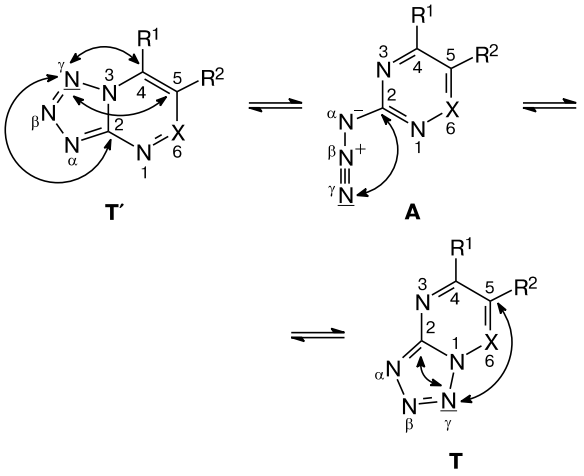
There is a class of hetaryl azides, whose cyclization can give two cyclic forms. For example, 2-azidopyrimidines **A** ($X = CR^3$, $R^1 \neq R^3$) containing various substituents in positions 4 and 6 of the azine ring can transform into tetrazole isomers **T** ($X = CR^3$, $R^1 \neq R^3$) and **T′** ($X = CR^3$, $R^1 \neq R^3$) (Scheme 1). In the most part of cases,

* Dedicated to the Academician of the Russian Academy of Sciences S. M. Aldoshin on the occasion of his 60th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 0519—0526, February, 2013.

X-ray diffraction analysis is used for the unambiguous determination of the route of azido group cyclization and establishment of the structure of tetrazolo[1,5-*a*]pyrimidines **T** (X = CR^3, $R^1 \neq R^3$) and **T´** (X = CR^3, $R^1 \neq R^3$). However, this method is not suitable for studying the dynamics of the transition of one isomeric form to another in solutions. On the contrary, ^1H and ^{13}C NMR spectroscopy allows one to directly observe azido-tetrazole rearrangements in a series of 2-azidopyrimidines, but often does not allow the structure of the formed isomers to be determined unambiguously because of the low content of hydrogen and carbon atoms in the structures of heterocycles of this class.

Scheme 1



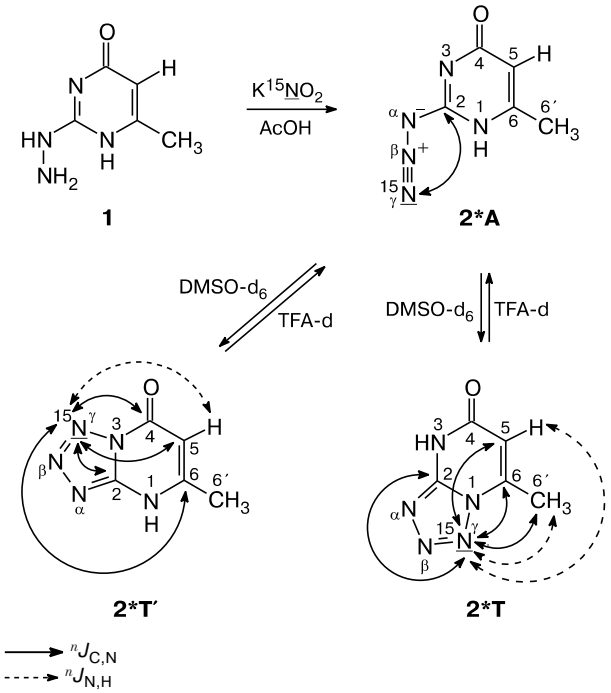
Other examples for similar compounds are 3-azido-1,2,4-triazine derivatives **A** (X = N), which are capable of cyclizing to form two cyclic isomers of tetrazolo[5,1-*c*]-[1,2,4]triazines **T´** (X = N) and tetrazolo[1,5-*b*][1,2,4]-triazines **T** (X = N) (see Scheme 1). It has previously[18] been shown that the selective introduction of the ^{15}N isotope in the γ-position of the azido group (N = ^{15}N) of open form **A** (X = N) makes it possible to detect open and cyclic forms and unambiguously establish the structures of tetrazolo-1,2,4-triazines from an analysis of chemical shifts of signals in the 1D ^{15}N NMR spectra and spin-spin coupling constants (SSCCs) $^{13}C—^{15}N$ ($J_{C,N}$). For example, the formation of tetrazolo[1,5-*b*][1,2,4]triazines **T** (N = ^{15}N, X = N) was confirmed by two $^{13}C—^{15}N$ constants in the 1D ^{13}C NMR spectra. In the case of cyclization of compound **A** (N = ^{15}N, X = N) to tetrazolo[5,1-*c*]-[1,2,4]triazines **T´**, $^nJ_{C,N}$ should be detected for all carbon atoms of the azine fragment (see Scheme 1).

In this approach, the potential of the $^1H—^{15}N$ SSCCs ($J_{N,H}$), which can be observed even in samples with the natural abundance of the ^{15}N isotope,[19] is not observed. The selective introduction of the ^{15}N label significantly facilitates the measurement of long-range $^1H—^{15}N$ SSCCs (for example, $^4J_{N,H}$ through four covalent bonds), substantially decreases requirements for the amount of the substance necessary for analysis, and makes it possible to apply $J_{N,H}$ for the determination of structures of more complicated organic molecules.

In this work, a complex approach based on the combined use of $^{13}C—^{15}N$ and $^1H—^{15}N$ SSCCs was proposed with the purpose for searching for the most efficient methods of investigation of the azido-tetrazole equilibrium for ^{15}N-labeled 2-azidopyrimidines as examples.

One of the methods for inclusion of the ^{15}N isotope into the structure of 2-azidopyrimidines is based on the interaction of 2-hydrazinopyrimidines with labeled nitrous acid generated from $K^{15}NO_2$ in an acidic medium.[18] Hetaryl azide **2*A** (enrichment in ^{15}N is 86%) containing the isotope label in the γ-position of the azido group was obtained by the same method from compound **1** (Scheme 2).

Scheme 2



An alternative approach to the synthesis of 2-azidoazines is based on ring opening in the corresponding tetrazole derivatives. In this case, to introduce the ^{15}N atom into the azide group/tetrazole fragment, it is necessary to use labeled 5-aminotetrazole, which builds up the azine fragment.[16,18] The ^{15}N isotope can be included into position 2 of 5-aminotetrazole by the treatment of aminoguanidine with nitrous acid or by the interaction of ^{15}N-aminoguanidine **3*** with HNO_2.[18] The combination of these two

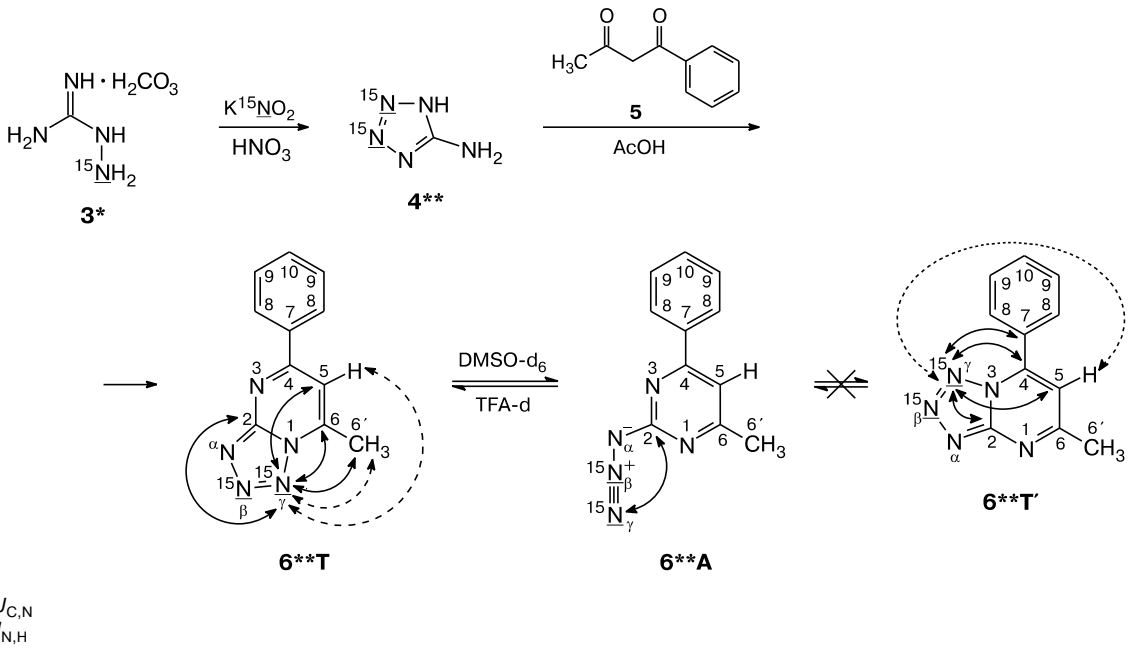
approaches allowed one to synthesize [$^{15}N_2$]-5-aminotetrazole **4**** containing simultaneously two isotope labels in the heterocycle. The further condensation of compound **4**** with benzoylacetone **5** afforded tetrazolo[1,5-*a*]pyrimidine **6**T** including the ^{15}N isotope in the β- and γ-positions of the azido group (86% enrichment in each ^{15}N atom, Scheme 3). It should be mentioned that earlier [2-^{15}N]-5-aminotetrazole containing one isotope label was applied to the preparation of unsubstituted ^{15}N-tetrazolo-[1,5-*a*]pyrimidines and ^{15}N-tetrazolo[1,5-*b*][1,2,4]triazines.[18,20] However, in this case, a mixture of ^{15}N isotopomers was formed at the β- and γ-positions of the azido group. The use of compound **4**** makes it possible to obtain samples of ^{15}N-tetrazolo[1,5-*a*]pyrimidines with the homogeneous isotope composition.

Pyrimidine derivatives **2*A** and **6**T** were studied by the NMR method in DMSO and TFA solutions, which make it possible to shift the azido-tetrazole equilibrium towards the tetrazole isomer or azide form, respectively. In addition, the ring-chain tautomerism of sample **2*A** was additionally studied in various mixtures of DMSO and TFA. The signals from the ^1H and ^{13}C nuclei were assigned using 2D ^1H—^{13}C HMBC and HMQC NMR experiments.

An analysis of the NMR spectra of sample **2*A** in a DMSO solution (Fig. 1, *a*) showed that the azide form undergoes spontaneous cyclization to form simultaneously two cyclic isomers **2*T´** and **2*T**. This conclusion was drawn on the basis of chemical shifts of the $^{15}N_\gamma$ nuclei (Table 1), which were detected in the range characteristic of tetrazoloazines.[18,21,22] An analysis of the integral intensities of signals in the 1D ^1H and ^{15}N NMR spectra made it possible to monitor the mutual transformation of two isomeric forms of compound **2***. Immediately after the dissolution of the production of the reaction of hetarylhydrazine **1** with labeled nitrous acid, the ratio of isomers **2*T** and **2*T´** was 92 : 8 (Table 2). Further the concentration of compound **2*T´** increased rapidly and changed to 49 : 51 already after incubation at 30 °C within 24 h (Table 2).

The 2D ^1H—^{15}N HMBC spectrum of a mixture of the tetrazole isomers for compounds **2*T** and **2*T´** contained cross-peaks between the labeled nitrogen atom ($^{15}N_\gamma$) and protons of the azine fragment, which directly indicated the long-range ^1H—^{15}N spin-spin interactions between the corresponding nuclei. However, a considerable broadening of the ^1H NMR signals of compound **2*T** probably caused by chemical exchange processes did not allow us to analyze the value of observed $J_{N,H}$ and conclude about the structure of the tetrazole isomers formed by the cyclization of azide **2*A**. Amplitude modulated 1D ^1H spin-echo experiments with selective inversion of the ^{15}N nuclei were used for the quantitative measurement of the observed ^1H—^{15}N SSCCs. The measured values of long-range $J_{N,H}$ (see Table 1) allowed us to establish the structures of compounds **2*T** and **2*T´** (see Scheme 2). For example, the ^1H—^{15}N spin-spin interaction at the H(5) proton and the protons of the methyl group at the C(6´) atom prove that

Scheme 3



Note. For compounds **6**T**, **6**A**, and **6**T´**, $^nJ_{C,N}$ and $^nJ_{N,H}$ are shown for the $^{15}N_\gamma$ atom only.

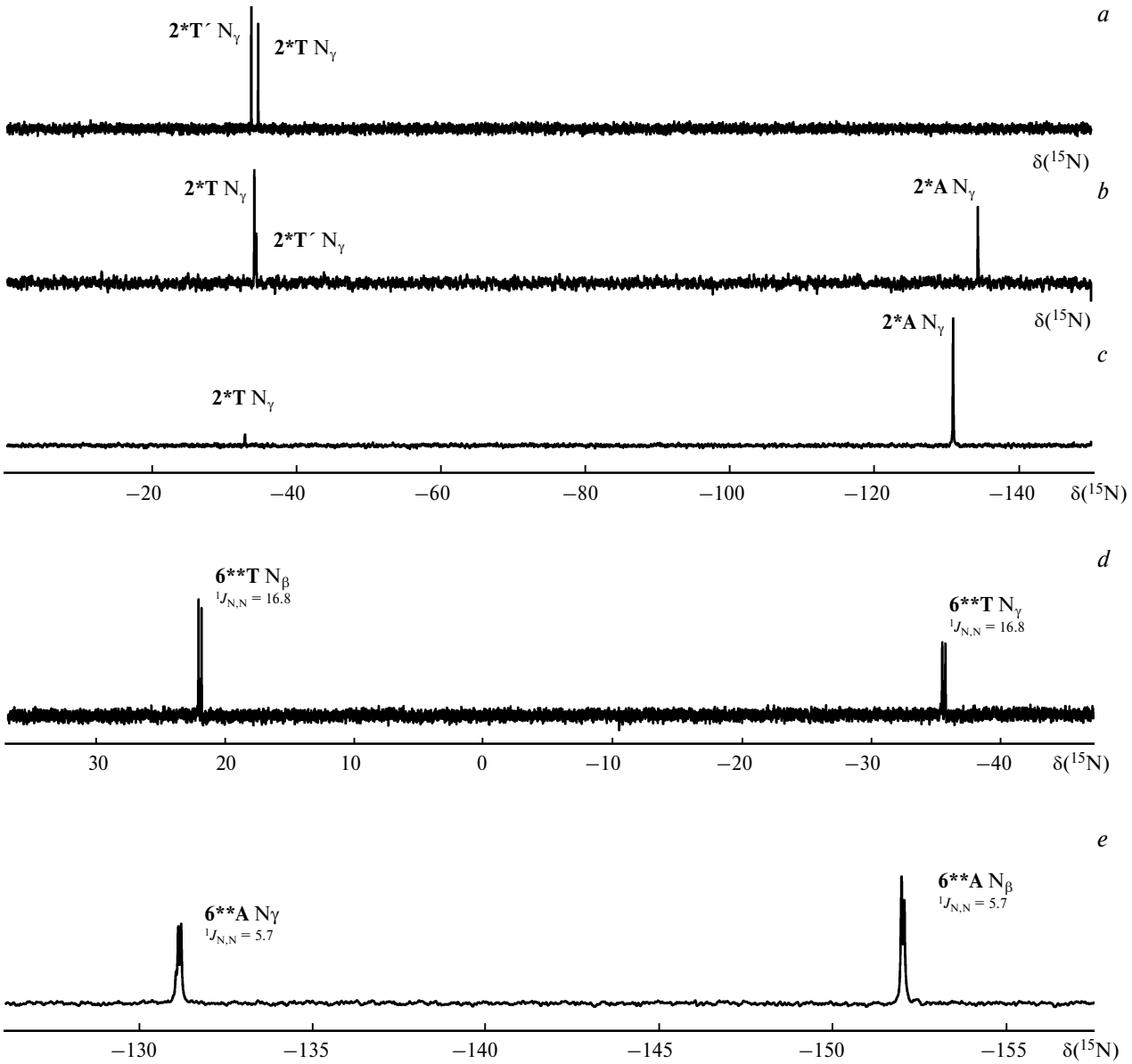


Fig. 1. 1D ^{15}N NMR spectra of compounds **2*** 24 h after dissolution in DMSO (*a*), in a DMSO—TFA (2 : 3) mixture (*b*), and in TFA (*c*) and of compounds **6**** in DMSO (*d*) and TFA (*e*).

the cyclization of compound **2*A** to tetrazole isomer **2*T** involves the N(1) atom of the pyrimidine ring. In the case of structure **2*T´**, the annelation mode for the tetrazole ring is confirmed by the observation of the single $^4J_{N,H}$ between the H(5) proton and N_γ nitrogen atom.

The conclusion about the structures of compounds **2*T** and **2*T´** is well consistent with the experimental data on the analysis of the $^{13}C—^{15}N$ coupling constants. Thus, in the 1D ^{13}C NMR spectrum of a mixture of tetrazolo-[1,5-*a*]pyrimidines for the C(2), C(4), C(5), and C(6) signals of compound **2*T´** exhibits spin-spin interactions with the $^{15}N_\gamma$ nucleus (Table 3, see Scheme 2). The interaction between the $^{13}C(4)$ and $^{15}N_\gamma$ atoms ($^2J_{C,N} = 2.1$ Hz) proves that the N(3) atom participates in tetrazole isomer formation. In the case of isomer **2*T**, $J_{C,N}$ was observed only for the signals of two C(6) and C(6´) atoms (see Table 3). The expected constants at C(2) and C(5) were not detected because of a considerable broadening of the corresponding ^{13}C NMR signals, which is induced by the dynamic transformation of the cyclic forms into each other and a possible influence of prototropic tautomerism for compounds **2*T** and **2*A**. However, the presence of the spin-spin interaction between the carbon atom of the methyl group $^{13}C(6´)$ and the $^{15}N_\gamma$ atom ($^3J_{C,N} = 0.8$ Hz) unambiguously confirms the structure of cyclic isomer **2*T** (see Scheme 2).

Table 1. Chemical shifts of the ^1H and ^{15}N nuclei and the $J_{H,H}$, $J_{N,H}$, and $J_{N,N}$ SSCC for compounds **2*** and **6****

Compound	Solvent	^1H (δ, J/Hz)[a]			^{15}N (δ, J/Hz)[b]	
		H(5)	Me	Other signals	N_γ/N_β	Other signals
2*T´[c]	DMSO	5.94 ($^4J_{N,H}$ = 0.6)	2.38 ($^4J_{H(5),H(6')}$ = 0.8)	—	−33.8 (N_γ)	−259.0 (N(1)); −136.9 (N(3))
	DMSO—TFA (3 : 1)	5.81	2.33	—	−33.8 (N_γ)	—
	DMSO—TFA (2 : 3)	5.67 ($^4J_{N,H}$ = 0.6)	2.22		−34.5 (N_γ)	—
2*T	DMSO	6.30[d] ($^4J_{N,H}$ = 0.6)	2.62 ($^4J_{N,H}$ = 0.2, $^4J_{H(5),H(6')}$ = 1.3)	—	−34.7 (N_γ)	−155.5 (N(1))
	DMSO—TFA (3 : 1)	6.17	2.56	—	−34.8 (N_γ)	—
	DMSO—TFA (2 : 3)	6.05 ($^4J_{N,H}$ = 0.6)	2.47 ($^4J_{N,H}$ = 0.2)	—	−34.2 (N_γ)	—
	TFA	6.22 ($^4J_{N,H}$ = 0.7)	2.51 ($^4J_{N,H}$ = 0.2)	—	−32.9 (N_γ)	−154.9 (N(1))
2*A[e]	DMSO—TFA (2 : 3)	6.34	2.24	—	−134.4 (N_γ)	—
	TFA	6.33	2.24[f]		−131.0 (N_γ)	−228.6 (N(1)); −159.6 (N(3))
6T**	DMSO	8.15 ($^4J_{N_\gamma,H}$ = 0.82, $^5J_{N_\beta,H}$ = 0.09)	2.99 ($^4J_{N_\gamma,H}$ = 0.20, $^5J_{N_\beta,H}$ = 0.08, $^4J_{H(5),H(6')}$ = 1.0)	8.34 (H(8)); 7.65 (H(9)); 7.66 (H(10))	−35.6 (N_γ), 22.0 (N_β) ($^1J_{N_\gamma,N_\beta}$ = 16.8)	−140.8 (N(1)); −126.7 (N(3))
6A**	TFA	7.70	2.65[f]	8.15 (H(8)); 7.48 (H(9)); 7.62 (H(10))	−131.3 (N_γ), −151.9 (N_β) ($^1J_{N_\gamma,N_\beta}$ = 5.7)	−214.4 (N(1)); −136.6 (N(3))

[a] The ^1H—^{15}N SSCCs ($J_{N,H}$) were measured using 1D experiments including amplitude-modulated spin-echo with ^{15}N nuclear selective inversion. The total time delay in the spin-echo experiments (the time of magnetization evolution for $J_{N,H}$) was 0.6 and 1.2 s for compounds **2*** and **6****, respectively. The experimental error in the measured values of $J_{N,H}$ does not exceed 0.05 and 0.03 Hz, respectively.

[b] The chemical shifts of the $^{15}N_\gamma$ and $^{15}N_\beta$ signals were measured in the 1D ^{15}N NMR spectra. Other signals were assigned at the natural content of the ^{15}N isotope using the 2D ^1H—^{15}N HMBC spectra.

[c] No signals of compound **2*T´** in TFA were observed.

[d] The signal is broadened.

[e] No signals of compound **2*A** in DMSO and DMSO—TFA (3 : 1) were observed.

[f] The $^4J_{H(5),H(6')}$ value was not measured because of a large half-width of the corresponding signals in the spectrum.

In spite of the fact that isomerization of tetrazoles **2*T** and **2*T´** to each other proceeds through structure **2*A**, no azido form was observed in a DMSO solution. Thus, it can be concluded that, under these conditions, the concentration of isomer **2*A** does not exceed 0.1%. For the stabilization of azide **2*A**, deuterated trifluoroacetic acid was added to a solution of a mixture of compounds **2*T** and **2*T´**. The DMSO : TFA volume ratio was varied from 9 : 1 to 2 : 3. The highest concentration of open form **2*A** (29%) was observed for the highest content of TFA in a solution (see Table 2). The change in the parameters of chemical exchange between diverse isomeric forms of compound **2*** induced by acid addition resulted in a significant broadening of the half-width of the $^{13}C(2)$ and $^{13}C(5)$ signals of compound **2*T´** and made it possible to measure $J_{C,N}$ for these nuclei (see Table 3). It is noteworthy that the chemical shifts of the ^{13}C NMR signals of compounds **2*T** and **2*T´** changed insignificantly upon the addition of TFA to DMSO, which facilitated the assignment of the signals of these compounds at different TFA concentrations.

An analysis of the NMR spectra of sample **2*A** in a TFA solution revealed that, under these conditions, azide form **2*A** is predominant and only **2*T**, whose concentration is 4%, of the tetrazole isomers exists in the solution (see Table 2). The structure of compound **2*A** was confirmed by the observation of the spin-spin interaction between the C(2) carbon and N_γ nitrogen nuclei ($^3J_{C,N}$ = = 0.6 Hz). In addition, the signal from the $^{15}N_\gamma$ atom (see Table 1) was observed in the region characteristic of azidoazines.[18,21,22] It was impossible to detect and measure the ^{13}C—^{15}N coupling constants for compound **2*T** in TFA because of the low concentration of this isomeric form. However, the amplitude-modulated spin-echo experi-

Table 2. Azido-tetrazole equilibrium of compounds **2*** and **6****

Compound	Solvent	Ratio of isomers **T** : **T´** : **A**
2*	DMSO[a]	92 : 8 : 0[b]
	DMSO[c]	49 : 51 : 0[d]
	DMSO—TFA (9 : 1)	50 : 50 : 0
	DMSO—TFA (3 : 1)	49 : 51 : 0
	DMSO—TFA (3 : 2)	49 : 51 : 0
	DMSO—TFA (1 : 1)	51 : 47 : 2
	DMSO—TFA (2 : 3)	47 : 24 : 29
	TFA	4 : 0 : 96[e]
6**	DMSO	100 : 0 : 0
	TFA	0 : 0 : 100

[a] The ratio of isomers was determined immediately after dissolution.

[b] Only one isomer **2*T** has been observed earlier[23] immediately after the dissolution of the compound in DMSO.

[c] The ratio of isomers 1 day after dissolution.

[d] The ratio of isomers **2*T** : **2*T´** equal to 52 : 48 has earlier[23] been observed after incubation at 100 °C in a DMSO solution within 5 h.

[e] Isomer **2*T** was not earlier detected in TFA.[23]

ments revealed the spin-spin interaction with the $^{15}N_{\gamma}$ nucleus at the H(5) proton and the protons of the methyl group at the C(6´) atom, which proved the structure of tetrazolo[1,5-*a*]pyrimidine **2*T**. It should be emphasized that the amplitudes of the long-range $^{1}H—^{15}N$ SSCCs measured for compound **2*T** in DMSO and TFA solutions coincide within the experimental error (see Table 1). It is also important that the results on studying the azido-tetrazole equilibrium of compound **2A** using an analysis of the $^{1}H—^{15}N$ and $^{13}C—^{15}N$ SSCCs supplement the earlier published data[23] (see Table 2), and the experiments in DMSO—TFA mixtures made it possible to reliably detect in solutions already three tautomeric forms: **2T´**, **2T**, and **2A**.

The obtained sample of compound **6**T** was studied by the NMR method in DMSO and TFA solutions. The 1D ^{15}N NMR spectrum of compound **6**T** recorded in DMSO exhibits two doublets with the SSCC $J_{N,N} \approx 16.8$ Hz, which was confirmed by the introduction of two isotope ^{15}N labels in the N_{γ} and N_{β} positions of the tetrazole fragment (see Fig. 1, *d*, Table 1). In this case, the signals from the $^{15}N_{\gamma}$ (δ −35.6) and $^{15}N_{\beta}$ (δ 22.0) nuclei were detected in the region characteristic of condensed tetrazaloazines.[18,21,22] In addition, the 2D $^{1}H—^{15}N$ HMBC spectrum of compound **6**T** contained cross-peaks between the labeled nitrogen atoms and the protons of the azine fragment caused by the long-range $^{1}H—^{15}N$ coupling constants, which directly indicated the cyclic structure of the compound.

The exact structure of compound **6**T** in a DMSO solution was determined by an analysis of the $^{1}H—^{15}N$ SSCCs. The use of the spin-echo experiments with ^{15}N nuclear selective inversion made it possible to observe the long-range $J_{N,H}$ between the protons of the C(6´)H_3 methyl group and two labeled nitrogen atoms ($^{4}J_{N_{\gamma},H} \approx \approx 0.20$ Hz, $^{5}J_{N_{\beta},H} \approx 0.08$ Hz, see Table 1). The obtained results show that the condensation of $^{15}N_2$-aminotetrazole **4**** with benzoylacetone **5** gives tetrazolopyrimidine **6**T**, since spin—spin interactions $^{6}J_{N_{\gamma},H(6')}$ and $^{6}J_{N_{\beta},H(6')}$ should be absent in the case of formation of alternative structure **6**T´** (see Scheme 3). This result also indicates the efficiency of an analysis of $^{4}J_{N,H}$ and $^{5}J_{N,H}$ as a method of determination of azole cycle annelation to the azine fragment in tetrazolo[1,5-*a*]pyrimidines.

The quantitative measurement of the $^{13}C—^{15}N$ SSCCs in a DMSO solution for compound **6**T** confirmed the conclusions about the structure of this substance obtained by an analysis of the $^{1}H—^{15}N$ constants. Thus, $^{13}C—^{15}N$ spin—spin interaction was observed for the C(2), C(5), C(6), and C(6´) atoms (see Scheme 3, Table 3). In the case of formation of alternative structure **6**T´**, the $^{13}C—^{15}N$ coupling constants should be detected for the C(4) and C(7) signals, and the $^{13}C—^{15}N$ constants at the C(6´) atom should be absent.

The chemical shifts of the $^{15}N_{\gamma}$ and $^{15}N_{\beta}$ signals (δ −131.3 and −151.9, respectively) in a TFA solution indicates the complete rearrangement of tetrazole isomer **6**T** to open form **6**A** (see Fig. 1, *e*, Table 1). Signal assignment in the 1D ^{15}N NMR spectrum of compound **6**A** was performed on the basis of the earlier published data.[18,21,22] The absence of the $^{1}H—^{15}N$ SSCCs in the 1D ^{1}H NMR spectrum and the presence of the single $^{13}C—^{15}N$ constant in the ^{13}C NMR spectrum unambiguously confirms the structure of azide **6**A**.

Thus, we showed the efficiency of the complex analysis of the $^{13}C—^{15}N$ and $^{1}H—^{15}N$ coupling constants in studying the azido-tetrazole tautomerism in selectively ^{15}N-labeled azidoazines for examples of pyrimidines **2** and **6**. It was demonstrated that the measurement and analysis of $J_{C,N}$ makes it possible to unambiguously establish the structure of the azide and tetrazole forms in solution, although requires significant concentrations of the studied substances. At the same time, the use of $J_{N,H}$ provides information on the structure of weakly populated (minor) isomeric forms. For instance, an analysis of the $^{1}H—^{15}N$ spin-spin interaction confirmed the structure of isomer **2*T**, whose population in a TFA solution is ~4%. The high sensitivity of ^{1}H NMR spectroscopy compared to that of ^{13}C NMR spectroscopy at the natural abundance makes it possible to reliably measure the heteronuclear $^{1}H—^{15}N$ SSCCs with a significantly lower amplitude (see Tables 1 and 3) and within a considerably shorter time that it is required for measuring the $^{13}C—^{15}N$ SSCCs (minutes and hours, respectively). Summating the aforesaid, we may conclude that the combined application of the $^{13}C—^{15}N$ and $^{1}H—^{15}N$ SSCCs in investigation of isomerization of azidoazines to tetrazolo-

Table 3. Chemical shifts of the ^{13}C nuclei and the $J_{C,N}$ SSCC of compounds **2*** and **6****

Compound	Solvent	^{13}C (δ, J/Hz)[a]				
		C(2)	C(4)	C(5)	C(6)	Other signals
2*T´[b]	DMSO	151.53 ($^2J_{C,N}$ = 2.4)	154.38 ($^2J_{C,N}$ = 2.1)	98.66 ($^3J_{C,N}$ = 0.8)	154.96 ($^4J_{C,N}$ = 0.4)	19.27 (Me)
	DMSO—TFA (3 : 1)	151.31 ($^2J_{C,N}$ = 2.4)	154.24 ($^2J_{C,N}$ = 2.1)	98.39 ($^3J_{C,N}$ = 0.8)	154.54[c] ($^4J_{C,N}$ = 0.4)	18.65 (Me)
	DMSO—TFA (2 : 3)	150.59	154.47	98.32	154.70[c]	17.90 (Me)
2*T	DMSO	150.98[c,d]	161.27[c,d]	109.10[c,d]	145.31[c] ($^2J_{C,N}$ = 3.4)	16.33 (Me) ($^3J_{C,N}$ = 0.8)
	DMSO—TFA (3 : 1)	150.78 ($^2J_{C,N}$ = 2.7)	160.99[c]	108.91 ($^3J_{C,N}$ = 0.7)	145.06 ($^2J_{C,N}$ = 3.4)	15.77 (Me) ($^3J_{C,N}$ = 0.8)
	DMSO—TFA (2 : 3)	149.64	161.58	108.15	146.15	14.99 (Me)
	TFA	—[e]	—[e]	108.34	148.31	14.35 (Me)
2*A[f]	DMSO—TFA (2 : 3)	157.26	173.55	103.81	160.73	17.54 (Me)
	TFA	157.61 ($^3J_{C,N}$ = 0.6)[g]	173.10	102.86	160.84	16.91 (Me)
6T**	DMSO	155.50 ($^2J_{C,N_\gamma}$ = 2.6, $^2J_{C,N_\beta}$ = 2.5)	164.38	110.02 ($^3J_{C,N_\gamma}$ = 0.6, $^4J_{C,N_\beta}$ = 0.5)	147.16 ($^2J_{C,N\gamma}$ = 3.1)	17.24 (Me) ($^3J_{C,N_\gamma}$ = 0.7, $^4J_{C,N_\beta}$ = 0.2) 135.80 (C(7)); 128.59 (C(8)); 129.74 (C9)); 132.68 (C(10))
6A**	TFA	156.42 ($J_{CN\gamma}$ = 0.6,[g] $J_{CN\beta}$ = 0.7)[g]	172.64	111.89	162.46	18.09 (Me); 131.36 (C(7)); 128.53 (C(8)); 129.23 (C(9)); 135.44 (C(10))

[a] The $^{13}C—^{15}N(7)$ SSCCs ($J_{C,N}$) were measured using the nonlinear approximation of the line shapes in the 1D ^{13}C NMR spectra recorded with ^{15}N selective decoupling and without it. The experimental error in measured $J_{C,N}$ values does not exceed 0.15 Hz.

[b] No signals of compound **2*T´** in TFA were observed.

[c] The signal is broadened.

[d] The signal was assigned upon the addition of TFA.

[e] The signal was not observed because of the low concentration of the corresponding isomer.

[f] No signals of compound **2*A** were observed in DMSO and DMSO—TFA (3 : 1).

[g] The $J_{C,N}$ values were measured using 1D ^{13}C NMR experiments including amplitude-modulated spin-echo with ^{15}N nuclear selective inversion. The total time delay in spin-echo experiments (the time of magnetization evolution for $J_{C,N}$) was 0.4 s. The experimental error in the measured $J_{C,N}$ values does not exceed 0.15 Hz.

azines makes it possible to study these processes more exactly and efficiently.

Experimental

^1H, ^{13}C, and ^{15}N NMR spectra were recorded on an Avance 700 spectrometer (Bruker) equipped with a triple resonance probe (^1H,^{13}C,^{15}N) using DMSO-d_6 and DMSO-d_6—TFA-d mixtures as solvents and Me_4Si (^{13}C, ^1H) and $MeNO_2$ (^{15}N) as internal and external standard, respectively. Chemical shifts in the ^1H, ^{13}C, and ^{15}N NMR spectra of solutions in TFA-d were obtained relative to the signals of the residual protons of the COOH group (δ 11.50), Me_4Si, and $MeNO_2$, respectively. The NMR spectra of compound **2*** in DMSO-d_6 and DMSO-d_6—TFA-d mixtures were measured at 30 °C, the NMR spectra of compound **6**** in DMSO-d_6 were recorded at 42 °C, and the NMR spectra of compounds **2*** and **6**** in TFA-d were detected at 27 °C.

Two earlier developed methods[18] were used to measure the $^{13}C—^{15}N$ coupling constants: the method of nonlinear approximation of line shapes in the 1D ^{13}C NMR spectra detected with ^{15}N nuclear selective decoupling and without it and the method of quantitative SSCCs measurement in amplitude-modulated spin-echo 1D ^{13}C experiments accumulated with selective inversion of ^{15}N nuclear magnetization in the spin-echo sequence and without it.[18] The $^1H—^{15}N$ coupling constant values were quantitatively measured using amplitude-modulated spin-echo 1D ^1H experiments. Delays (2×Δ) in the range from 0.4 to 1.2 s were used in the spin-echo sequence (the total time of magnetization evolution for $J_{C,N}$ and $J_{N,H}$). Adiabatic impulses

(WURST-20) with a length of 10—20 ms and a width of the inversion range of ~1 kHz (14 ppm for ^{15}N) were used for selective decouping and ^{15}N nuclear inversion.

High-resolution mass spectra were measured with a Finnigan LTQ FT mass spectrometer (Germany) equipped with a superconducting magnet with a field intensity of 7 T and an Ion Max electrosprayer.

Potassium ^{15}N-nitrite[18] (with 86% ^{15}N enrichment) and ^{15}N-aminoguanidine[24] **3**** (with 86% ^{15}N enrichment) were synthesized according to earlier described procedures. Benzoylacetone (**5**) was purchased from Aldrich.

2-Hydrazino-6-methylpyrimidin-4-one (1) was synthesized by an earlier described method.[25]

[^{15}N]-2-Azido-6-methylpyrimidin-4-one (2*A). A solution of $K^{15}NO_2$ (0.08 g, 0.93 mmol in 1 mL of H_2O) was added to a solution of compound **1** (0.1 g, 0.71 mmol in 3.00 mL of AcOH) cooled to 5 °C. The reaction mixture was stirred for 2 h at 5 °C, H_2O (10 mL) was added, and the mixture was treated with AcOEt (3×7 mL). The organic layers were separated, dried with anhydrous Na_2SO_4, and evaporated. Compound **2*A** was obtained in a yield of 0.054 g (50%), m.p. 240—242 °C. Found: *m/z* 153.05399 $[M + H]^+$. $C_5H_5N_4{}^{15}NO$. Calculated: $[M + H]^+ = 153.0542$.

[$^{15}N_2$]-5-Aminotetrazole monohydrate (4).** A 5 *M* solution of $K^{15}NO_2$ (1 mL) was added to a suspension of [^{15}N]-aminoguanidine carbonate **3*** (0.75 g, 5.5 mmol) in 1 mL of 5 *M* nitric acid at the temperature ≤40 °C. Sodium acetate (1.5 g) was added to the obtained yellow solution, and the mixture was refluxed for 5 min. The solution was cooled, and the precipitate formed was filtered off and dried. Compound **4**** was obtained in a yield of 0.38 g (66%), m.p. 200 °C. Found: *m/z* 88.04034 $[M + H]^+$. $CH_3N_3{}^{15}N_2$. Calculated: $[M + H]^+ = 88.04074$.

[$^{15}N_2$]-7-Methyl-5-phenyltetrazolo[1,5-*a*]pyrimidine (6T).** Benzoylacetone (**5**) (0.39 g, 2.41 mmol) was added to a solution of compound **4**** (0.25 g, 2.38 mmol) in AcOH (2 mL). The reaction mixture was refluxed for 1.5 h. After cooling, the precipitate formed was filtered off and dried. Compound **6**T** was obtained in a yield of 0.25 g (49%), m.p. 179—183 °C. Found: *m/z* 214.08730 $[M + H]^+$. $C_{11}H_9N_3{}^{15}N_2$. Calculated: $[M + H]^+ = 214.08768$.

This work was financially supported by the Russian Foundation for Basic Research (Project Nos 10-03-01007-a and 12-03-31476), the Ministry of Education and Science of the Russian Federation (agreement Nos 14.A18.21.0806 and 14.A18.21.0830), and the Program for Development of the Ural Federal University (grants for support of young scientists).

References

1. H.-J. Schäfer, G. Rathgeber, K. Dose, Y. Kagawa, *FEBS Lett.*, 1989, **253**, 264.
2. F. Boulay, P. Dalbon, P. V. Vignais, *Biochemistry*, 1985, **24**, 7372.
3. L. P. Kotra, P. P. Wang, M. G. Bartlett, K. Shanmuganathan, Z. Xu, S. Cavalcanti, M. G. Newton, C. K. Chu, *J. Org. Chem.*, 1997, **62**, 7267.
4. T. Koudriakova, K. K. Monouilov, K. Shanmuganathan, L. P. Kotra, F. D. Boudinot, E. Cretton-Scott, J.-P. Sommadossi, R. F. Schinazi, C. K. Chu, *J. Med. Chem.*, 1996, **39**, 4676.
5. L. P. Kotra, K. K. Manouilov, E. Cretton-Scott, J.-P. Sommadossi, F. D. Boudinot, R. F. Schinazi, C. K. Chu, *J. Med. Chem.*, 1996, **39**, 5202.
6. Y. O. El-Khoshien, *Phosphorus*, *Sulfur*, *Silicon*, 1998, **139**, 163.
7. Th. Kappe, A. Pfaffenschlager, W. Stadlbauer, *Synthesis*, 1989, 666.
8. V. Ya. Pochinok, L. F. Avramenko, P. S. Grigorenko, V. N. Skopenko, *Russ. Chem. Rev.*, 1976, **45**, 183.
9. V. Ya. Pochinok, L. F. Avramenko, P. S. Grigorenko, V. N. Skopenko, *Russ. Chem. Rev.*, 1975, **44**, 481.
10. M. Tišler, *Synthesis*, 1973, 123.
11. N. B. Smirnova, I. Ya. Postovskii, N. N. Vereshchagina, I. B. Lundina, I. I. Mudretsova, *Chem. Heterocycl. Compd.* (*Engl. Transl.*), 1970, **4**, 130 [*Khim. Geterotsikl. Soedin.*, 1968, 167].
12. V. A. Ershov, I. Ya. Postovskii, *Chem. Heterocycl. Compd.* (*Engl. Transl.*), 1971, **5**, 668 [*Khim. Geterotsikl. Soedin.*, 1971, 711].
13. H. Takeuchi, K. Watanabe, *J. Phys. Org. Chem.*, 1998, **11**, 478.
14. B. Chattopadhyay, C. I. Rivera Vera, S. Chuprakov, V. Gevorgyan, *Org. Lett.*, 2010, **12**, 2166.
15. J. H. Boyer, M. S. Chang, R. F. Reinich, *J. Org. Chem.*, 1960, **25**, 286.
16. E. N. Ulomskii, T. S. Shestakova, S. L. Deev, V. L. Rusinov, O. N. Chupakhin, *Russ. Chem. Bull.* (*Int. Ed.*), 2005, **54**, 726 [*Izv. Akad. Nauk*, *Ser. Khim.*, 2005, 713].
17. D. E. Macfalane, D. C. B. Mills, P. C. Srivastava, *Biochemistry*, 1982, **21**, 544.
18. S. L. Deev, Z. O. Shenkarev, T. S. Shestakova, O. N. Chupakhin, V. L. Rusinov, A. S. Arseniev, *J. Org. Chem.*, 2010, **75**, 8487.
19. D. G. Davis, W. C. Agosta, D. Cowburn, *J. Am. Chem. Soc.*, 1983, **105**, 6189.
20. T. S. Shestakova, S. L. Deev, E. N. Ulomsky, V. L. Rusinov, O. N. Chupakhin, O. A. D´yachenko, O. N. Kazheva, A. N. Chekhlov, P. A. Slepukhin, M.I. Kodess, *Russ. Chem. Bull.* (*Int. Ed.*), 2006, **55**, 2071 [*Izv. Akad. Nauk*, *Ser. Khim.*, 2006, 1993].
21. P. Cmoch, L. Stefaniak, G. A. Webb, *Magn. Reson. Chem.*, 1997, **35**, 237.
22. P. Cmoch, J. W. Wiench, L. Stefaniak, G. A. Webb, *J. Mol. Struct.*, 1999, **510**, 165.
23. C. Temple, Jr., W. C. Coburn, Jr., M. C. Thorpe, J. A. Montgomery, *J. Org. Chem.*, 1965, **30**, 2395.
24. O. N. Chupakhin, E. N. Ulomsky, S. L. Deev, V. L. Rusinov, *Synth. Commun.*, 2001, **31**, 2351.
25. L. E. Brady, R. M. Herbst, *J. Org. Chem.*, 1959, **24**, 922.

Received December 13, 2012